1: J Am Vet Med Assoc 1991 Nov 15;199(10):1423-7

Development of a genetically engineered vaccine against feline leukemia virus infection.

Kensil CR, Barrett C, Kushner N, Beltz G, Storey J, Patel U, Recchia J, Aubert A, Marciani D.

Cambridge Biotech Corporation, Worcester, MA 01605.

A genetically engineered subunit vaccine against FeLV infection was developed. The protective immunogen in the vaccine was a purified recombinant protein containing the entire amino acid sequence of FeLV subgroup A gp70 envelope protein. The optimal adjuvant was determined to be a highly purified saponin, QS-21, derived from Quillaja saponaria Molina. A vaccine formulation containing the recombinant protein, QS-21, and aluminum hydroxide was tested in specific-pathogen-free kittens and was shown to induce neutralizing antibodies as well as appreciable antibody responses to native gp70 by enzyme immunoassay and protein (western) immunoblot analysis and of whole virus preparations.

PMID: 1666096 [PubMed - indexed for MEDLINE]

Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex.

Kensil CR, Patel U, Lennick M, Marciani D.

Cambridge Biotech Corporation, Worcester, MA 01605.

Saponins were purified from Quillaja saponaria Molina bark by silica and reverse phase chromatography. The resulting purified saponins were tested for adjuvant activity in mice. Several distinct saponins, designated QS-7, QS-17, QS-18, and QS-21, were demonstrated to boost antibody levels by 100-fold or more when used in mouse immunizations with the Ag BSA and beef liver cytochrome b5. These purified saponins increased titers in all major IgG subclasses. To determine optimal dose in mice for adjuvant response, QS-7 and QS-21 were tested in a dose-response study in intradermal immunization with BSA in mice; for both of these purified saponins, adjuvant response (determined by stimulation of ELISA titers to BSA) neared maximum at doses of 5 micrograms and was shown to plateau up to the highest dose tested, 80 micrograms. These purified saponins vary considerably in their toxicity, as assessed by lethality in mice; the main component, QS-18, being the most toxic. Saponins QS-7 and QS-21 showed no or very low toxicity in mice, respectively. None of these saponins stimulated production of reaginic antibodies. The monosaccharide composition of these saponins showed similar but distinct compositions with all four containing fucose, xylose, galactose and glucuronic acid. Predominant differences were observed in the quantities of rhamnose, arabinose, and glucose. Monomer m.w. (determined by size exclusion HPLC) were determined to range from 1800 to 2200.

PMID: 1987271 [PubMed - indexed for MEDLINE]

The Journal of Immunology

The Journal of Immunology, Vol 146, Issue 2 431-437, Copyright © 1991 by American Association of Immunologists

ARTICLES

Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex

CR Kensil, U Patel, M Lennick and D Marciani
Cambridge Biotech Corporation, Worcester, MA 01605.

Saponins were purified from Quillaja saponaria Molina bark by silica and reverse phase chromatography. The resulting purified **saponin**s were tested for adjuvant activity in mice. Several distinct **saponin**s, designated QS-7, QS-17, QS-18, and QS-21, were demonstrated to boost antibody levels by 100-fold or more when used in mouse immunizations with the Ag BSA and beef liver cytochrome b5. These purified **saponin**s increased titers in all major IgG subclasses. To determine optimal dose in mice for adjuvant response, QS-7 and QS-21 were tested in a dose- response study in intradermal immunization with BSA in mice; for both of these purified **saponin**s, adjuvant response (determined by stimulation of ELISA titers to BSA) neared maximum at doses of 5 micrograms and was shown to plateau up to the highest dose tested, 80 micrograms. These purified **saponin**s vary considerably in their toxicity, as assessed by lethality in mice; the main component, QS-18, being the most toxic. **Saponin**s QS-7 and QS-21 showed no or very low toxicity in mice, respectively. None of these **saponin**s stimulated production of reaginic antibodies. The monosaccharide composition of these **saponin**s showed similar but distinct compositions with all four containing fucose, xylose, galactose and glucuronic acid. Predominant differences were observed in the quantities of rhamnose, arabinose, and glucose. Monomer m.w. (determined by size exclusion HPLC) were determined to range from 1800 to 2200.

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- Gilewski, T., Adluri, S., Ragupathi, G., Zhang, S., Yao, T.-J., Panageas, K., Moynahan, M., Houghton, A., Norton, L., Livingston, P. O. (2000). Vaccination of High-Risk Breast Cancer Patients with Mucin-1 (MUC1) Keyhole Limpet Hemocyanin Conjugate plus QS-21. *Clin Cancer*

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L1: Entry 6 of 6 File: USPT Apr 7, 1998

US-PAT-NO: 5736139
DOCUMENT-IDENTIFIER: US 5736139 A

TITLE: Treatment of Clostridium difficile induced disease

DATE-ISSUED: April 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kink; John A.	Madison	WI		
Thalley; Bruce S.	Madison	WI		
Stafford; Douglas C.	Madison	WI		
Firca; Joseph R.	Vernon Hills	IL		
Padhye; Nisha V.	Madison	WI		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Ochidian Pharmaceuticals, Inc.	Madison	WI			02

APPL-NO: 8/ 480604
DATE FILED: June 7, 1995

PARENT-CASE:
This application is a Continuation-in-Part of application Ser. No. 08/422,711 filed Apr. 14, 1995, which is a Continuation-in-Part of application Ser. No. 08/405,496 filed Mar. 16, 1995, which is a Continuation-in-Part of application Ser. No. 08/329,154 filed, Oct. 24, 1994, which is a Continuation-in-Part of application Ser. No. 08/161,907, filed on Dec. 2, 1993, now U.S. Pat. No. 5,601,823, which is a Continuation-in-Part of application Ser. No. 07/985,321, filed Dec. 4, 1992, which is a Continuation-in-Part of application Ser. No. 429,791, filed Oct. 31, 1989, now issued as U.S. Pat. No. 5,196,193 on Mar. 23, 1993.

INT-CL: [6] A61K 39/395, C07K 16/12
US-CL-ISSUED: 424/164.1; 424/167.1, 530/389.1, 530/389.5
US-CL-CURRENT: 424/164.1; 424/167.1, 530/389.1, 530/389.5
FIELD-OF-SEARCH: 424/164.1, 424/167.1, 530/389.1, 530/389.5

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5080895	January 1992	Tokoro	424/85.8
☐	5196193	March 1993	Carroll	424/85.8
☐	5268295	December 1993	Serrero	435/252.3

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ART-UNIT: 186
PRIMARY-EXAMINER: Eisenschenk; Frank C.
ATTY-AGENT-FIRM: Medlen & Carroll, LLP

ABSTRACT:

The present provides neutralizing antitoxin directed against C. difficile toxins. These antitoxins are produced in arian species using soluble recombinant C. difficile toxin proteins. The avian antitoxins are designed so as to be orally administrable in therapeutic amounts and may be in any form (i.e., as a solid or in aqueous solution). Solid forms of the antitoxin may comprise an enteric coating. These antitoxins are useful in the treatment of humans and other animals intoxicated with at least one bacterial toxin. The invention further provides vaccines capable of protecting a vaccinated recipient from the morbidity and mortality associated with C. difficile infection. These vaccines are useful for administration to humans and other animals at risk of exposure to C. difficile toxins.

28 Claims, 55 Drawing figures

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L1: Entry 6 of 6 File: USPT Apr 7, 1998

US-PAT-NO: 5736139
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TITLE: Treatment of Clostridium difficile induced disease

DATE-ISSUED: April 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kink; John A.	Madison	WI		
Thalley; Bruce S.	Madison	WI		
Stafford; Douglas C.	Madison	WI		
Firca; Joseph R.	Vernon Hills	IL		
Padhye; Nisha V.	Madison	WI		

US-CL-CURRENT: 424/164.1; 424/167.1, 530/389.1, 530/389.5

CLAIMS:

We claim:
1. A method of treating Clostridium difficile disease, comprising:
a) providing:
i) a subject exposed to Clostridium difficile exhibiting symptoms comprising diarrhea; and
ii) avian antibody reactive with Toxin A of Clostridium difficile, said antibody in a therapeutic amount that is orally administrable, and
b) administering said antibody orally to said subject under conditions such that said subject ceases to exhibit symptoms and treatment can be terminated.
2. The method of claim 1, wherein said subject exhibits long-term survival beyond the treatment period.
3. The method of claim 1, wherein said avian antibodies reacts with the A-6 interval of Toxin A and wherein said A-6 interval has the sequence set forth in SEQ ID NO:29.
4. The method of claim 1, further comprising oral administration of an avian antibody reactive with Toxin B of Clostridium difficile.
5. The method of claim 4, wherein said avian antibodies react with the B-3 interval of Toxin B and wherein said B-3 interval has the sequence set forth in SEQ ID NO:30.
6. A method of treating Clostridium difficile disease, comprising:
a) providing:
i) a subject,
ii) a first avian antitoxin directed against Clostridium difficile Toxin A, wherein said antitoxin neutralizes Clostridium difficile Toxin A in vivo, and
iii) a second avian neutralizing antitoxin directed against Clostridium difficile Toxin B, wherein said antitoxin neutralizes Clostridium difficile Toxin B in vivo;
b) mixing said first and second antitoxin to create a therapeutic mixture; and
c) administering said therapeutic mixture orally to said subject.
7. The method of claim 6 further comprising the step of, prior to step c), processing said therapeutic mixture to improve its enteric stability.
8. The method of claim 7, wherein said processing comprises combining said

therapeutic mixture with nutritional formula.
9. The method of claim 7 wherein said processing comprises encapsulating said antitoxins of said therapeutic mixture.
10. The method of claim 9 wherein said encapsulating step comprises overcoating with an enteric film.
11. The method of claim 6 wherein said subject has been exposed to either Clostridium difficile toxin A or toxin B prior to administration of said antitoxin.
12. The method of claim 11 wherein said subject is suffering from the symptoms of bacterial intoxication and said administering results in the substantial elimination of said symptoms.
13. The method of claim 12 wherein said symptoms comprise diarrhea.
14. The method of claim 6 wherein said subject has not been exposed to either Clostridium difficile toxin A or toxin B prior to administration of said antitoxin.
15. The method of claim 6, wherein said first avian antitoxin is directed against a portion of Clostridium difficile Toxin A sequence SEQ ID NO:6.
16. The method of claim 15, wherein said portion of Clostridium difficile Toxin A comprises a protein sequence selected from the group comprising SEQ ID NOS:7, 8 and 29.
17. The method of claim 6, wherein said second avian antitoxin is directed against a portion of Clostridium difficile Toxin B sequence SEQ ID NO: 10.
18. The method of claim 17, wherein said portion of Clostridium difficile Toxin B comprises a protein sequence selected from the group comprising SEQ ID NOS:20, 21 and 30.
19. A method of treating Clostridium difficile disease, comprising:
a) providing:
i) a subject,
ii) a first avian antitoxin directed against Clostridium difficile Toxin A, wherein said antitoxin neutralizes Clostridium difficile Toxin A in vivo, and
iii) a second avian neutralizing antitoxin directed against Clostridium difficile Toxin B, wherein said antitoxin neutralizes Clostridium difficile Toxin B in vivo;
b) mixing said first and second antitoxin to create a therapeutic mixture;
c) encapsulating said therapeutic mixture; and
d) administering said encapsulated therapeutic mixture orally to said subject.
20. The method of claim 19 wherein said encapsulating step comprises overcoating with an enteric film.
21. The method of claim 19 wherein said subject has been exposed to either Clostridium difficile toxin A or toxin B prior to administration of said antitoxin.
22. The method of claim 21 wherein said subject is suffering from the symptoms of intoxication and said administering results in the substantial elimination of said symptoms.
23. The method of claim 22 wherein said symptoms comprise diarrhea.
24. The method of claim 19 wherein said subject has not been exposed to either Clostridium difficile toxin A or toxin B prior to administration of said antitoxin.
25. The method of claim 19, wherein said first avian antitoxin is directed against a portion of Clostridium difficile Toxin A sequence SEQ ID NO:6.
26. The method of claim 25, wherein said portion of Clostridium difficile Toxin A comprises a protein sequence selected from the group comprising SEQ ID NOS:7, 8 and 29.
27. The method of claim 19, wherein said second avian antitoxin is directed against a portion of Clostridium difficile Toxin B sequence SEQ ID NO: 10.
28. The method of claim 27, wherein said portion of Clostridium difficile Toxin B comprises a protein sequence selected from the group comprising SEQ ID NOS:20, 21 and 30.